DOI:36108/NJE/2002/91.0120

NIGERIAN JOURNAL OF ENTOMOLOGY (2002) 19:15-21

Toxicity of Hexanolic Extract of *Piper guineense* Schum and Thonn (Piperaceae) Seed Oil to Larvae of *Aedes aegypti* (L).

ANYAELE, O.O.[1], * AMUSAN, A.A.S.,[2] OKORIE T.G.[1] and OKE O.A.[2]
[1]Department of Zoology University of Ibadan, Ibadan, Nigeria.
[2]Department of Biological Sciences, College of Natural Science University of Agriculture, P.M.B. 2240, Abeokuta Nigeria.

*To whom correspondence should be addressed.
E-mail:amusan@unaab.edu.ng

(Accepted 02 October, 2001)

ABSTRACT

Hexanolic extract of *Piper guineense* Schum and Thonn (Piperaceae) was tested for acute toxicity on third instar larvae of *Aedes aegypti* reared in the Advanced Entomology Laboratory of the University of Ibadan, Nigeria. Five graded concentrations 0.9ppm, 1.7ppm, 2.5ppm, 3.3ppm and 4.1ppm, were tested on the larvae. Effects of two physical parameters namely; sunlight and ultra-violet radiation on the potency of the extract was also investigated. The extract showed a high level of toxicity on the 3rd instar larvae of *A. aegypti*. LC50 of *P.guineense* on the larvae was1.7 ± 0.84ppm.The highest concentration of 4.1ppm killed 77% of the population within an hour and 95% in 24 hours while at the least concentration of 0.9ppm no death was recorded but killed 16% of the larvae in 24 hours. The potency of *P. guineense* on the larvae was drastically reduced when exposed to sunlight. However, there was no significant difference between the potency of the U-V exposed extract and the untreated, rather the raw data showed some level of activation by ultraviolet radiation.

INTRODUCTION

The solution to the problem of mosquito-borne diseases lies ultimately with effective anti-mosquito measures. This is because mosquitoes are regarded as the weak link in the chain of malaria transmission. Such anti-mosquito measure, according to WHO (1993), includes elimination of breeding sites, killing of mosquito larvae, destruction of adult mosquitoes and prevention of man-vector contact.

Mosquito larvae could be controlled either by using biological methods or chemical method otherwise known as larvicidal method. The chemical method is particularly effective in well defined and limited breeding sites. However, many synthetic chemicals that have been used in the control of mosquitoes have been

reported to cause ecological imbalance manifested by pollution of the environment, which may lead to reduction in the ozone layer that shields the earth from the ultraviolet rays of the sun. Botanical pesticides are suitable alternatives to these synthetic chemicals, because they are rapidly degraded by microorganisms or are unstable under sunlight (Wink, 1993). *Piper guineense* is one of such botanicals.
The present work is therefore aimed at investigating the toxicity of *P. guineense* seed oil to 3rd instar larvae of *Aedes aegypti* reared in the Laboratory.

MATERIALS AND METHODS

Sand particles and debris from the breeding sites of mosquitoes in disused tyres in the maintenance Unit, University of Ibadan, were collected and soaked in water for a day. Hatching of viable eggs actually commenced after 24 hours of soaking. Identification of *A. aegypti* larvae was done using Hopkins(1951). The hatched larvae at various stages of development were collected using a pipette into clean water in bright coloured enamel plates (for easy visibility), where they were reared to pupae. The active swimming pupae were collected into 250ml open bottles and placed in a cage where they were left to emerge as adult mosquitoes.

The cages of about 40cm by 40cm size were made from light wooden frame with sides of mosquito netting, (black netting). The base is made of wooden board and one side of the cage is provided with sleeve for taking materials in and out of the cage.

Caged rabbits, that have been shaved to enable biting assess, were used as source of blood meal for the adult mosquitoes, and were allowed to feed on the rabbit for fifteen minutes. Eggs laid on moist filter paper placed round the sides(inside) of a 500ml beaker were collected, dried and stored in an incubator at 31 ± 2°C. All the third instar larvae required for the study were obtained from these stored eggs.

Dry fruits of *P. guineense* obtained from Oje market Ibadan Nigeria, were washed and sun-dried. These were pulverized using an electric grinder. Extraction of the oil with N-hexane was done using Soxhlet extractor. The extract was then concentrated using a Rotary evaporator, which removed the hexane component leaving behind a brown viscous oil that was used for the study.

One milli-liter of the extract was measured and emulsified with 0.003ml or 3 drops of Tween-80 from a needle tip. The emulsified extract was then made up to 1 litre with distilled water to form 1000ppm stock solution. From this stock solution, serial concentrations were prepared. The range of concentrations chosen were determined by preliminary test that found the lowest concentration that kill all the larvae in a test and the highest concentration that kill none. The range arrived were 0.9ppm, 1.7ppm, 2.5ppm, 3.3ppm and 4.1ppm.

The stock solution was divided into 3 places. One part for the preparation of one set of the concentration was exposed to sunlight for 2, 4 and 8 hours. Another set was exposed to a U-V lamp (Gallen Kamp LH 530) with a peak output at 366nm for 2, 4 and 8 hours. The distance of the light source from solutions was about 30cm .

Shallow containers were used to enhance penetration of the U-V Light. The third part was administered without any treatment. At the end of each interval the exposed extracts were then used to make the respective serial concentration as in the untreated control.

Toxicity Bioassay

From each dilution 250ml were introduced into labeled specimen bottles, of 500ml capacity. Forty third instar larvae of *A. aegypti* were introduced into each bottle. Each treatment had four replicates. Mortalities were recorded at intervals of 12,24,48,72 and 96 hours. The 96-hour LC50 (concentration that killed 50 % of the population) of the extract on *A. aegypti* third instar larvae were estimated with the 96 hour mortality of the various sets of the different concentrations using Probit regression graph. Data obtained from the processes described above were then subjected to analysis of variance to confirm differences at 5% level of significance. Also Duncan's Multiple Range test was used to determine whether there were significant differences between treated and untreated means.

Where mortality was obtained in the control, Abbot's (1925) formula was used to correct percentage mortality.

RESULTS AND DISCUSSION

The mean percentage mortality data of the bioassay on the third instar larvae is presented in Tables 1-3.There was a significant difference (P>0.001) in % mortality between treated and untreated larvae. The mean lethal concentration (LC50) at 96 hours as presented in Table 1 ranges between 1.12ppm,(untreated) and 4.01ppm, when exposed to 8 hrs sunlight, and 0.66ppm and 1.30ppm when exposed to U-V radiation.

TABLE 1: LC50 at 96 hours treatment with *P. guineense* Extract.

Treatments on Extract	LC50 of Larvae
Untreated extract	1.12ppm
2 hrs sunlight	1.45ppm
4 hrs sunlight	1.95ppm
8 hrs sunlight	4.07ppm
2 hrs U-V	0.90ppm
4 hrs U-V	1.30ppm
8 hrs U-V	0.66ppm

Tables 2&3 show results obtained when *P. guineense* extract was exposed to U-V radiation and sunlight respectively, before subsequent exposure of the larvae. Duncan's test (Table 4) showed a significant difference between the means of the untreated and treated (P>0.05). The hexanolic extract of *P. guineense* showed larvicidal properties on the test organisms (3rd instar larvae of *A. aegypti*). The highest concentration, 4.1ppm killed 77% of the population within an hour of the test period and 95% mortality recorded in 24 hours. The least concentration of 0.99ppm did not kill any of the larvae within an hour of the test period, however 16% mortality was recorded in 24 hours. The results obtained in the present study is very remarkable considering the fact that most of the earlier works done on effect of *Piper* Fruit oil on insects has been on stored products insects. From such works of for example Su (1977) on Rice weevils and Ivbijaro (1989) on *Callosobruchus maculatus, Piper* fruit oil was reported to be very toxic to the developmental stages of the insects. This may account for the larvicidal effect it showed on *A. aegypti.*

After 24 hours the rate of mortality decreased significantly. Virtually no activity occurred at 96 hours. Therefore, the toxicity of the Piper fruit extract shows non-persistence over a long period of 96 hours. This result is remarkable considering Wink's (1993), assertion that active pesticidal principles of plant origin do not persist in the environment for a long time. This result corroborates the findings that, *Piper* fruit possess insecticidal properties (Su, 1977); Miyakado (1979) and Ivbijaro (1989). The larvicidal property of *Piper* is a valuable discovery because the larvae are regarded as the weakest link in disease transmission (WHO 1993). The insecticide activities of *P. guineense* has been attributed to piperine and piperic acid acting in synergism with some other compounds (Su, 1977). Miyakado (1979), also identified pipericide as being responsible for the insecticidal activity, while Okwute (1978), identified guineensine as the active ingredient.

The result of investigation into the degrading effects of physical parameters as presented in Table 1, compares the mean lethal concentration (LC50) at 96 hours between the untreated and the various levels of treatments of the extract before subsequent exposure of the larvae. Considering the effect of sunlight on the potency of the extract, this experiment demonstrated that there was a significant effect of sunlight exposure on the potency at 0.05 significance level. As the sunlight exposure period increases from 2-4-8 hours the larvicidal properties decreases. Sunlight significantly reduced the potency of *P. guineense* hexanolic extract at 4 and 8 hours exposure. The evidence of photodecomposition of *P. guineense* hexane extract was first observed during the exposure period when the orange coloured emulsified stock solution turned colourless after about $2^1/_2$ hours exposure to sunlight. This result is remarkable considering Winks' (1993), assertion that active pesticidal activities of plant origin are unstable under sunlight. Duncan and Sturrock (1983) also reported that synthetic molluscicides such as pentachlorophenol are degraded by sunlight.

TABLE 2: Effects of U-V radiation (% mortality in Time) on hexane extract of *P. guineense* on activity against *A. aegypti* larvae

Conc.	12 hrs				24 hrs				48 hrs				72 hrs				96 hrs			
PPm	n	u2	u4	u8	n	n2	u4	u8	n	u2	u4	u8	n	u2	u4	u8	n	u2	u4	u8
4.1	80	80	100	62.5	95	95	100	100	95	95	100	100	97.5	97.5	100	100	100	100	100	100
3.3	70	70	92.5	35	87.5	87.5	87.5	97.5	92.5	92.5	97.5	90	97.5	97.5	97.5	90	100	100	97.5	90
2.5	22	62.5	90	50	60	85	97.5	77.5	84	85	97.5	97.5	86	87.5	97.5	100	86	87.5	97.5	100
1.7	4	22.5	67.5	2.5	26	27.5	77.5	60	48	47.5	77.5	80	61	65	77.5	90	72	65	77.5	90
0.9	0	12.5	35	7.5	16	15	42.5	30	30	37.5	42.5	60	30	47.5	45	70	46	50	45	70
Ctrl.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

KEY:

n	=	no treatment	u4	=	4 hrs U-V exposure
u2	=	2 hrs U-V exposure	u8	=	8 hrs U-V exposure

TABLE 3: Effects of sunlight exposure (% mortality in Time) on hexane extract of *P. guineense*. on activity against *A. aegypti* larvae

Conc.	12 hrs				24 hrs				48 hrs				72 hrs				96 hrs			
PPm	n	S2	S4	S8	n	S2	S4	S8	n	S2	S4	S8	n	S2	S4	S8	n	S2	S4	S8
4.1	80	20	22.5	17.5	95	77.5	67.5	45	95	87.5	77.5	50	97.5	92.5	80	50	100	100	92.5	50
3.3	70	22.5	17.5	0	87.5	67.5	35	20	92.5	77.5	50	30	97.5	80	60	37.5	100	80	60	37.5
2.5	34	10	17.5	0	60	50	40	17.5	84	50	42.5	20	88	60	42.5	20	86	60	44	30
1.7	18	0	0	5	26	17.5	15	5	48	20	17.5	10	64	20	17.5	10	72	30	20	10
0.9	12	0	0	0	16	5	0	0	30	10	05	0	30	15	5	0	46	17.5	5	0
Ctrl.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

KEY:

n	=	no treatment	S2	=	2 hrs Sunlight exposure
S4	=	4 hrs Sunlight exposure	S8	=	8 hrs Sunlight exposure

TABLE 4: Comparison of treatment means

Treatments	Concentrations
U.V radiation	
Untreated	8.00
2 hrs	8.50
4 hrs	8.90
8 hrs	9.00
LSD (5%)	1.3042
Sunlight exposure	
Untreated	8.000
2 hrs	7.600
4 hrs	3.600
8 hrs	3.600
LSD (5%)	1.6977

However, there was no significant effect of UV- radiation on the extract as Duncan's test showed no significant difference between the means of various treatments ($P>0.05$). Hence there was no observed decrease in potency, rather a slight activation was observed when compared with the effect of the untreated extract.

Ultraviolet radiation is a component of sunlight. Deductively, it could be said that the decomposition of *P. guineense* hexane extract by sunlight does not result from a single effect of ultra-violet radiation since its effects was even found to be activating, though insignificantly. Perhaps the degrading effect of sunlight could be attributed to the single or combined effect of other components like the visible light and heat.

CONCLUSION

Hexane extract of *P. guineense* could be used as larvicides in the control of mosquito larvae due to its high toxicity, environmental compatibility and non-persistence. *P. guineense* is readily available and easy to procure. However, further

research needs to be carried out in order to determine the mode of action and elucidate the active ingredients present in the plant.

REFERENCES

ABBOT, W.S. 1925. A method for computing the effectiveness of an insecticide. *Journal of Economic Entomology* 18: 265-267

DUNCAN, J. AND STURROCK. 1983. Laboratory evaluation of potential plant molluscicides *London School of Hygiene and Tropical Medicine. Plant Molluscicides*.12:15-21.

HOPKINS, G.H.E 1951. Mosquitoes of the Ethiopian region I – Larval Bionomics of Mosquitoes and Taxonomy of Culicine larvae. British museum (Nat. Hist.): London

IVBIJARO, M.F. 1989. The efficacy of seed oils of *Azadirachta indica* A. Juss and *Piper guineense* Schum and Thonn on the Control of *Callosobruchus maculatus* (F) *Insect Science & its application* 11 (2) :149-152.

MIYAKADO, M. NAKAYAMA, I., TOSHOKA, H. AND NATATONI, N.N. 1979. The Piperacae amide; structure of Pipericide, a new insecticidal amide from *Piper nigrum* (L). *Agricultural Biochemistry* 43: 1609-1611.

OKWUTE, S.U. 1978. Synthesis of amides from *P. guineense* Schum and Thonn A Ph.D. thesis submitted to the University of Ibadan 250 pp.

SU, H.C.F. 1977 Insecticidal properties of black pepper to rice weevils *Journal of Economic Entomology* 70 (1):18-23.

W.H.O. 1993 Malaria Update. *Bulletin of the World Health Organization* 70 (6): 801-807.

WINK, M. 1993. Production and application of Phytochemicals from an Agricultural Perspective. *Proceedings of Phytochemical Society of Europe. Phytochemical* and *Agriculture* 34:18-22.